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(71)(72) Applicant and Inventor: <b>HOLICK, Michael, F. [US/US];</b> 31 Bishop Lane, Sudbury, MA 01776 (US).			
(74) Agents: <b>ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein &amp; Fox, Suite 600, 1100 New York Avenue, N.W.,</b> Washington, DC 20005-3934 (US).			
(54) Title: <b>METHOD FOR DELIVERING ESTROGEN</b>			
(57) Abstract  The present invention relates to methods for the treatment or prevention of osteoporosis and the alleviation of the symptoms of menopause by the administration of an estrogen glycoside or estrogen orthoester glycoside.			

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## Method For Delivering Estrogen

### *Background of the Invention*

#### *Field of the Invention*

The invention is in the field of Medicinal Chemistry.

#### 5      *Related Art*

Menopause is defined as the final episode of menstrual bleeding in women. The term is also used colloquially to refer to the period of time encompassing the transitional period between the reproductive years up to and beyond the final episode of menstrual bleeding. For the purposes of this invention, reference to menopause will encompass this transitional period. Menopause is the result of the cessation of follicular development, which leads to a drop in the production of estradiol and other hormones. While 60% of estrogen formed in pre-menopausal women is in the form of estradiol, most of which is produced in the ovaries of ovulating women, post-menopausal ovaries produce a minimal amount of estrogen, with extraglandular tissues providing the majority of post-menopausal estrogen synthesis.

Symptoms associated with menopause include vasomotor instability (hot flashes), a decrease in breast size, atrophy of the urogenital epithelium and skin, and osteoporosis, a disease which is characterized by a marked loss of bone mass. Hot flashes may be accompanied by nervousness, anxiety, irritability and depression. Osteoporosis and the decrease in size of the female reproductive tract and breasts have been closely correlated to low levels of estrogen, while the pathology of hot flashes, though less well understood, may also be related to the decrease in estrogen production accompanying menopause.

The significant loss of bone mass which occurs at the time of menopause ultimately gives rise to osteopenia, which in turn gives rise to

spontaneous crush fractures of the vertebrae and fractures of the long bones. This disease is generally known as postmenopausal osteoporosis and presents a major medical problem, both in the United States and most other countries where the life-span of females reaches ages of at least 60 and 70 years. Generally the disease, which is often accompanied by bone pain and decreased physical activity, is diagnosed by one or two vertebral crush fractures with X-ray evidence of diminished bone mass. It is known that this disease is accompanied by diminished ability to absorb calcium, decreased levels of sex hormones, especially estrogen and androgens, and a negative calcium balance.

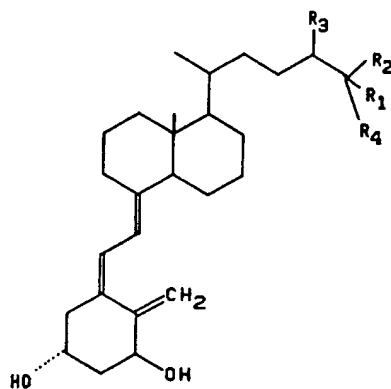
Methods for treating the disease have varied considerably but to date no really satisfactory treatment is yet being practiced. For example, calcium supplementation by itself has not been successful in preventing or curing the disease. Other treatments, for which variable results have again been reported, have included a combination of vitamin D in large doses, calcium and fluoride. The primary problem with this approach is that fluoride induces structurally unsound bone, called woven bone, and in addition, produces a number of side effects such as increased incidence of fractures and gastrointestinal reaction to the large amounts of fluoride administered.

Estrogen therapy is the most commonly used treatment of menopausal symptoms. Estrogen therapy ameliorates symptoms including hot flashes and atrophy of the urogenital epithelium and skin, and has positive effects on the incidence of osteoporosis.

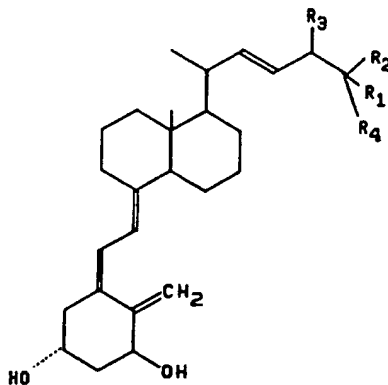
Premarin is the most widely prescribed estrogen that is used for the treatment of postmenopausal symptoms, including osteoporosis, in the United States. Premarin is obtained from pregnant mares' urine, and contains the sodium salts of water-soluble estrogen sulphates as well as a variety of estrogen derivatives. One of the reasons that Premarin is so popular is because of concern that unconjugated estradiol, when given orally, has a first pass effect on the liver. This effect can have potentially serious consequences including the increase of blood clotting factors which can increase the user's risk of developing blood clots.

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U.S. Patent No. 4,225,596 discloses methods for treating or preventing metabolic bone disease characterized by the loss of bone mass by administering at least one compound having the formulae (I) and (II):



(I)



(II)

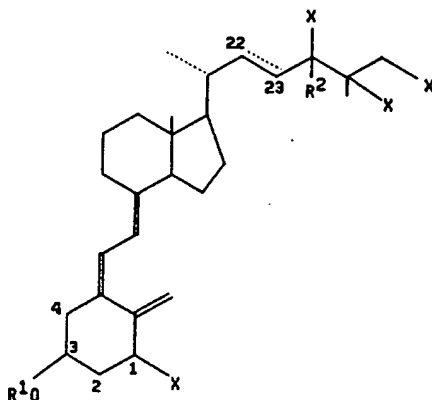
5

where R<sub>1</sub>, R<sub>2</sub> and R<sub>4</sub> are each selected from the group consisting of hydrogen, hydroxyl, lower alkyl, acyl and O-alkyl and R<sub>3</sub> is selected from the group consisting of hydrogen, hydroxyl, keto, lower alkyl, acyl and O-alkyl.

10

U.S. Patent No. 4,410,515 discloses the following compounds having Formula (III) which are active in maintaining calcium and phosphorus metabolism and are useful for treating hypocalcemia in animals:

-4-



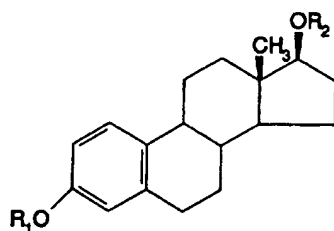
(III)

wherein the bond between positions C-22 and C-23 is single or double; R<sup>2</sup> is hydrogen, CH<sub>3</sub> or CH<sub>2</sub>CH<sub>3</sub>; X is selected from the group consisting of hydrogen and -OR<sup>1</sup>, where R<sup>1</sup> is hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue; with the proviso that at least one of the R<sup>1</sup> is glycosidic residue.

Fujimoto, *et al.* (Fujimoto *et al.*, *Experientia* 42:567-568 (1988)) reported the biochemical synthesis of 17 $\beta$ -estradiol-3-( $\beta$ -D-glucoside) and 17-( $\alpha$ -D-glucoside) by incubation of 17 $\beta$ -estradiol with ovarian tissues from a silkworm. Similarly, incubation of 17 $\alpha$ -estradiol 3-glucuronide with rabbit liver microsomes in the presence of uridine diphosphate glucose results in the synthesis of 17 $\alpha$ -estradiol-17-( $\beta$ -glucoside). (Williamson *et al.*, *Biochemistry* 8:45299-4304 (1969)). When 17 $\beta$ -estradiol-17-glucoside and 17 $\alpha$ -estradiol-17-glucoside were injected into normal women, the 3-glucuronide derivatives of each compound were recovered in the urine (Williamson *et al.*, *Canadian J. Biochemistry* 50:958-962 (1972)).

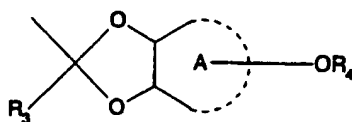
### Summary of the Invention

The present invention relates to a method for treating or preventing osteoporosis, as well as a method for alleviating the symptoms of menopause, in an animal having osteoporosis or susceptible to osteoporosis, or undergoing or having undergone menopause, comprising administering to the animal an effective amount of a compound having the Formula (IV):



(IV)

wherein  $R_1$  and  $R_2$  are independently hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue, or  $R_1$  or  $R_2$  is an orthoester glycoside moiety of the Formula (V):



(V)

wherein A represents a glycofuranosyl or glycopyranosyl ring;

$R_3$  is hydrogen, lower alkyl ( $C_1$ - $C_4$ ), aralkyl ( $C_7$ - $C_{10}$ ), or aryl, with the proviso that aryl is phenyl or phenyl substituted by chloro, fluoro, bromo, iodo, lower  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  alkoxy; or naphthyl;

$R_4$  is hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue; with the further proviso that at least one of  $R_1$  and  $R_2$  is either a glycosidic residue or an orthoester glycoside moiety.

### *Brief Description of the Figures*

Figure 1A shows a reverse phase HPLC (2% H<sub>2</sub>O/methanol, 1.5 ml/min) of <sup>3</sup>H-vitamin D<sub>3</sub>-glucoside after incubation with rat intestinal homogenate for 3 hours at 37°C. No <sup>3</sup>H-vitamin D<sub>3</sub> was formed.

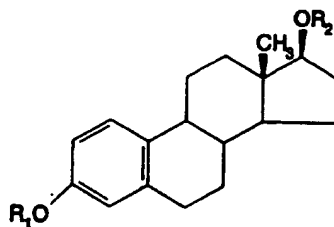
5 Figure 1B shows a reverse phase HPLC (2% H<sub>2</sub>O/methanol, 1.5 ml/min) of <sup>3</sup>H-vitamin D<sub>3</sub>-glucoside after incubation with rat kidney homogenate for 3 hours at 37°C. Note the formation of <sup>3</sup>H-vitamin D<sub>3</sub>.

Figures 2A and 2B show the serum vitamin D concentration in human subjects after 3250 nmoles of either vitamin D<sub>3</sub> (2A) or vitamin D<sub>3</sub>-glucoside (2B) was given orally.

10 Figure 3 shows the sequence of reactions for the synthesis of 17β-estradiol-3β-glucoside.

### *Description of the Preferred Embodiments*

The invention is related to the discovery that compounds having Formula (IV) are useful in treating and preventing osteoporosis, as well as in alleviating the symptoms associated with menopause:

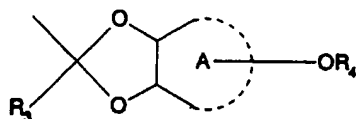


(IV)

wherein R<sub>1</sub> and R<sub>2</sub> are independently hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue, or R<sub>1</sub> or R<sub>2</sub> is an orthoester glycoside moiety of the Formula (V):



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(V)

wherein A represents a glycofuranosyl or glycopyranosyl ring;

R<sub>3</sub> is hydrogen, lower alkyl (C<sub>1</sub>-C<sub>4</sub>), aralkyl (C<sub>7</sub>-C<sub>10</sub>), or aryl, with the proviso that aryl is phenyl or phenyl substituted by chloro, fluoro, bromo, iodo, lower C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkoxy; or naphthyl;

R<sub>4</sub> is hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue;

with the further proviso that at least one of R<sub>1</sub> and R<sub>2</sub> is either a glycosidic residue or an orthoester glycoside moiety.

According to the invention, the symptoms of menopause which may be alleviated include atrophy of the urogenital epithelium and skin, decreased breast size, hot flashes and osteoporosis. Any animal which experiences any of these symptoms and which may benefit from the estradiol glycosides and orthoester glycosides of Formula IV may be treated according to the present invention. Preferred animals are of course humans, in particular, pre- or post-menopausal women. When administered to a pre-menopausal woman, it is possible to prevent osteoporosis. When administered to a post-menopausal woman, it is possible to reverse the adverse consequences of osteoporosis mentioned above, and arrest the further deterioration of the bones. Administration of these compounds during menopause can alleviate the occurrence of hot flashes, decreased breast size and atrophy of the urogenital tract and skin.

By glycosidic units are meant glycopyranosyl or glycofuranosyl, as well as their amino sugar derivatives. The residues may be homopolymers, random or alternating or block copolymers thereof. The glycosidic units have free hydroxy groups, or hydroxy groups acylated with a group R<sub>5</sub>-(C=O)-, wherein R<sub>5</sub> is hydrogen, lower C<sub>1-6</sub> alkyl, C<sub>6-10</sub> substituted or unsubstituted aryl or C<sub>7-16</sub> aralkyl. Preferably, the acyl groups are acetyl or propionyl. Other

preferred groups are where R<sub>5</sub> is phenyl, nitrophenyl, halophenyl, lower alkyl substituted phenyl, lower alkoxy substituted phenyl, and the like or benzyl, lower alkoxy substituted benzyl and the like.

5 The compounds useful in the practice of the invention contain at least one glycoside or orthoester glycoside residue at positions 3 or 17. They may contain, however, two glycoside or orthoester glycoside residues simultaneously. Preferably, the glycosides or orthoester glycosides are linked through the 1-carbon to the 3- or 17-position on estradiol.

10 The glycosides can comprise up to 20 glycosidic units. Preferred, however, are those having less than 10, most preferred, those having 3 or less than 3 glycosidic units. Specific examples are those containing 1 or 2 glycosidic units in the glycoside residue.

15 The glycopyranose or glycofuranose rings or amino derivatives thereof may be fully or partially acylated or completely deacylated. The completely or partially acylated glycosides are useful as defined intermediates for the synthesis of the deacylated materials.

20 Among the possible glycopyranosyl structures are glucose, mannose, galactose, gulose, allose, altrose, idose, or talose. Among the furanosyl structures, the preferred ones are those derived from fructose, arabinose or xylose. Among preferred diglycosides are sucrose, cellobiose, maltose, lactose, trehalose, gentiobiose, and melibiose. Among the triglycosides, the preferred ones may be raffinose or gentianose. Among the amino derivatives are N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, N-acetylneuraminic acid, D-glucosamine, lyxosylamine, D-galactosamine, and the like.

25 When more than one glycosidic unit is present on a single hydroxy group (i.e., di or polyglycosidic residues), the individual glycosidic rings may be bonded by 1-1, 1-2, 1-3, 1-4, 1-5 or 1-6 bonds, most preferably 1-2, 1-4 and 1-6. The linkages between individual glycosidic rings may be  $\alpha$  or  $\beta$ .

30 The water soluble glycosidic derivatives of the aforementioned compounds may be obtained according to the general methods set disclosed

in Holick, U.S. Patent 4,410,515, the contents of which are fully incorporated by reference herein. The estradiol glycosyl orthoester compounds may be obtained according to U.S. Patent 4,521,410, the contents of which are fully incorporated by reference herein. Preferred estradiol glycosyl orthoesters are  
5 3-estradiol-17 $\beta$ -( $\alpha$ -D-glucopyranosyl-1',2'-orthoacetate) and 17 $\beta$ -estradiol-3- $\beta$ -glucopyranosyl-1',2'-orthoacetate.

The compounds of the invention can be administered in any appropriate pharmaceutically acceptable carrier for oral, parenteral, or topical administration. They can be administered by any means that treats or prevents  
10 osteoporosis or alleviates the symptoms of menopause in animals, especially humans. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. For example, systemic daily dosage of 3-( $\beta$ -glucopyranosyl)-17 $\beta$ -estradiol is from about 0.001  
15 milligrams/kg to 100 milligrams/kg, preferably 0.01 to 1.0 milligrams per kg of body weight. Normally, from about 0.01 to 0.1 milligrams/kg per day of the glycoside or orthoester glycoside, in one or more dosages per day is effective to obtain the desired results. One of ordinary skill in the art can determine the optimal dosages and concentrations of other active estradiol,  
20 vitamin D glycoside and orthoester glycoside compounds with only routine experimentation.

The compounds can be employed in dosage forms such as tablets, capsules or powder packets, or liquid solutions, suspensions or elixirs for oral administration, as well as sterile liquid for formulations such as solutions or  
25 suspensions for parenteral use. The compounds could also be administered via topical patches, ointments, gels or other transdermal applications. In such compositions, the active ingredient will ordinarily be present in an amount of at least 1.0% by weight based upon the total weight of the composition, and not more than 90% by weight. An inert pharmaceutically acceptable carrier  
30 is preferably used. Among such carriers include 95% ethanol, vegetable oils, propylene glycols, saline buffers, etc.

It is well recognized that  $\beta$ -glucosidases do not exist in the small intestine because, if they did, humans would be able to digest cellulose as do ruminants. A study was conducted whereby  $^3\text{H}$ -vitamin  $\text{D}_3$ - $3\beta$ -glucoside was incubated with intestine and kidney homogenates. It was found that only the  
5 kidney homogenate was capable of removing the glucoside from vitamin  $\text{D}_3$ . None of the vitamin  $\text{D}_3$ - $3\beta$ -glucoside was metabolized in the intestinal homogenates. See Figure 1. Therefore, the same results for estradiol- $3\beta$ -glucoside would be expected since vitamin D and estradiol are both fat soluble sterols.

10 Since the small intestine does not contain  $\beta$ -glucosidases, it would not be expected that the liver would receive free estradiol coming from the intestine after the oral ingestion of estradiol-3-( $\beta$ -glucoside). Thus, this would lessen the first pass effect of estradiol-3-( $\beta$ -glucoside) when compared to estradiol itself.

15 *In vivo* rat studies have shown that when estradiol-3-( $\beta$ -glucoside) was given orally for two weeks to ovariectomized rats, estradiol was found in the circulation (Table 1). Thus, the non-specific  $\beta$ -glucosidases found in peripheral tissues appear to be responsible for the de-conjugation of estradiol-3-( $\beta$ -glucoside) to estradiol. As shown in Figure 1, a study with the vitamin  
20  $\text{D}_3$ - $3\beta$ -glucoside as a model compound was conducted which shows that  $\beta$ -glucosidase(s) in the kidney are able to de-conjugate the  $\beta$ -glucoside from the vitamin  $\text{D}_3$ - $3\beta$ -glucoside.

One of the potential side-effects of giving estradiol orally is its first pass biologic effect on the liver especially as it relates to increasing the  
25 synthesis of proteins associated with blood clotting. Since there is evidence that estradiol-3-( $\beta$ -glucoside) does not interact with the estradiol receptor, it would be expected that the estradiol-3-( $\beta$ -glucoside) would not have the same potency of activity in increasing blood clotting factors in the liver as estradiol itself.

30 De-conjugation of estradiol-3-( $\beta$ -glucoside) to estradiol will occur slowly in peripheral tissues, and therefore, will maintain circulating levels of

estradiol at a more physiologic level for a longer period of time. A study in humans with the vitamin D-3 $\beta$ -glucoside was conducted as a model compound. The blood level of vitamin D after an equimolar dose of vitamin D<sub>3</sub> and vitamin D<sub>3</sub>-3 $\beta$ -glucoside was compared. As can be seen in Figure 2, the blood levels of vitamin D<sub>3</sub> resulting from the de-conjugation of its glucoside is about 30% of that achieved when the same dose of vitamin D<sub>3</sub> was given orally. Thus, from this single dose vitamin D-glucoside study, there would not appear to be any hazard to the prolonged duration of action of the estradiol resulting from the slow de-conjugation of the estradiol-3-( $\beta$ -glucoside). A study in ovariectomized rats was also conducted to determine the blood level of estradiol and estrone after receiving three different doses of either estradiol or estradiol-3-( $\beta$ -glucoside) orally each day for two weeks. As can be seen in Table 1, the circulating estradiol levels in the rats receiving either 1, 10 or 15 nmol of estradiol-3-( $\beta$ -glucoside) were comparable or lower to the rats receiving the same doses of estradiol. Thus, estradiol-3-( $\beta$ -glucoside) will increase circulating concentrations of estradiol.

Based on the results in rats and humans with the model vitamin D-3 $\beta$ -glucoside and experience with giving estradiol-3-( $\beta$ -glucoside) to ovariectomized rats, there is no indication of potential additive or multiplicative toxicity. Indeed, in the study where oral administration of estradiol-3-( $\beta$ -glucoside) to ovariectomized rats was evaluated for a period of two weeks, it was found that the  $\beta$ -estradiol levels were either no different or lower compared to the animals that received unconjugated estradiol (Table 1). No untoward side-effects or vaginal bleeding was noted in either group of rats.

Having now generally described this invention, the same will be understood by reference to the following examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

### Example 1

#### *The synthesis of 17 $\beta$ -estradiol-3-( $\beta$ -glucoside)*

17 $\beta$ -Estradiol-3- $\beta$ -glucoside was synthesized as outlined in Figure 3. Estrone was coupled with acetobromoglucose in the presence of cadmium carbonate (CdCO<sub>3</sub>) as a catalyst following the procedure of Conrow and Bernstein (Conrow and Bernstein, *J. Org. Chem.* 36:863-870 (1971)). The  
5 estrone 3 $\beta$ -2'3',4',6'-tetra-O-acetyl-D-glucopyranoside (A) was the predominant product (> 60%) of the reaction and was obtained by crystallization. The melting point of the product was 212-214°C (reported mp  
10 212-216°C) (Conrow and Bernstein, *J. Org. Chem.* 36:863-870 (1971)). The IR and NMR spectra of this product matched that of reported values (Conrow and Bernstein, *J. Org. Chem.* 36:863-870 (1971)).

The estrone conjugate (A) was reduced with sodium borohydride at 0°C in methanol-THF. This reduction resulted in the stereo specific  
15 formation of the 17 $\beta$ -alcohol (Hobkirk et al., *J. Clin. Endocrinol.* 32:476-480 (1971)). The product (B) was obtained in almost quantitative yield after preparative TLC purification from silica plates (2:1 hexane-ethyl acetate).

Compound B was treated with methanolic ammonia at 4°C. Removal of the solvent and trituration with water produced a white crystalline solid,  
20 mp 210-220°C. The yield of the product (C), 17 $\beta$ -estradiol-3 $\beta$ -glucoside was quantitative. The HPLC analysis of the product (C<sub>18</sub>-column, 20% water in methanol, 0.5 ml/minute, monitored with a UV detector set at 254 nm) produced a single peak which demonstrated its homogeneity. Interpretation of NMR and mass spectrum data is given below.

## Example 2

### *Physico-Chemical Analysis of 17 $\beta$ -Estradiol-3 $\beta$ -Glucoside (C)*

The UV absorption spectrum for compound C in methanol had a typical spectrum for estradiol, wherein  $\lambda_{\max} = 280$  nm.

5 For the mass spectrum and NMR analysis of compound C, it was converted to its penta-O-acetyl derivative by treatment with pyridine-acetic anhydride-catalytic amount of 4,4'-dimethylaminopyridine. The reaction product was homogeneous on TLC and was purified by HPLC on a silica column with 5% isopropanol in n-hexane at 1 ml/minute. The isolated  
10 compound was analyzed by FABMS and NMR:

FABMS: 662.6, 643.5, 332.3, 331.3, 288.4.

NMR: (200 MHz;CDCl<sub>3</sub> solvent, TMS internal standard)  $\delta$  7.28 (overlapped with 7.26 peak of CHCl<sub>3</sub>, aromatic H);  $\delta$  6.85 (m, aromatic H, 2H),  $\delta$  5.15 (m, 4H, H-1', 2', 3', 4'),  $\delta$  4.56 (d, J=9 Hz; 1H, 17-CH);  $\delta$  4.2  
15 (m, 2H, 6'-CH<sub>2</sub>),  $\delta$  3.72 (m, 1H, 5'-CH);  $\delta$  2.85 (m, 2H, H-6-CH<sub>2</sub>);  $\delta$  2.3 and 2.2 (5 singlets, 15 H, 5-OAc-protons);  $\delta$  0.75 (s, 3H, 18-CH<sub>3</sub>).

## Example 3

### *In vivo Rat Studies*

20 *In vivo* rat studies were carried out where estradiol-3-( $\beta$ -glucoside) was given orally for two weeks to ovariectomized rats. Estradiol was detected in the circulation by radioimmunoassay, using a kit purchased from ICN Biomedical (Costa Mesa CA, 92626). The results are shown in Table 1.

Table 1				
Circulating concentration of 17 $\beta$ -estradiol and estrone in six ovariectomized rats per group that received an oral dose of either 17 $\beta$ -estradiol or 17 $\beta$ -estradiol-3 $\beta$ -glucoside (estradiol-glc) each day for two weeks.				
	Compounds	Dose (nmol)	Blood Concentrations	(pg/ml)
			Estradiol	Estrone
1.	control	0	3.2 $\pm$ 0.5	22.7 $\pm$ 3.6
2.	estradiol	1	3.5 $\pm$ 0.7	17.8 $\pm$ 2.0
3.	estradiol	10	8.7 $\pm$ 2.1	18.5 $\pm$ 2.9
4.	estradiol	15	46.8 $\pm$ 10.4	19.8 $\pm$ 7.0
5.	estradiol-glc	1	3.8 $\pm$ 0.7	12.2 $\pm$ 1.5
6.	estradiol-glc	10	7.7 $\pm$ 2.1	12.8 $\pm$ 1.5
7.	estradiol-glc	15	12.3 $\pm$ 3.4	11.5 $\pm$ 0.7

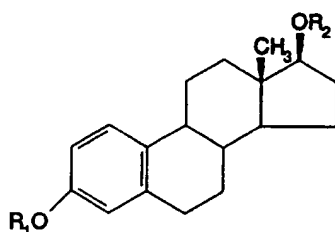
As can be seen in Table 1, the circulating estradiol levels in the rats receiving either 1, 10 or 15 nmol of estradiol-3-( $\beta$ -glucoside) were comparable or lower to the rats receiving the same doses of estradiol.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions without undue experimentation. All patents and publications cited herein are incorporated by reference in their entirety.

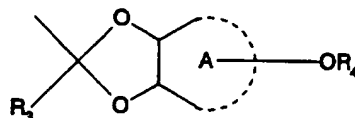


*What is Claimed is:*

1. A method for treating or preventing osteoporosis in an individual having osteoporosis or susceptible to osteoporosis, comprising administering to said individual an effective amount of a compound having the formula:



wherein  $R_1$  and  $R_2$  are independently hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue, or  $R_1$  or  $R_2$  is an orthoester glycoside moiety of the formula:



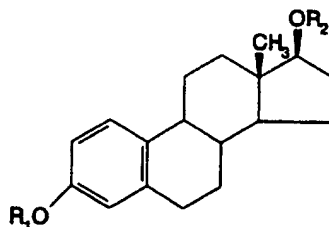
wherein A represents a glycofuranosyl or glycopyranosyl ring;

$R_3$  is hydrogen, lower alkyl ( $C_1$ - $C_4$ ), aralkyl ( $C_7$ - $C_{10}$ ), or aryl, with the proviso that aryl is phenyl or phenyl substituted by chloro, fluoro, bromo, iodo, lower  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  alkoxy; or naphthyl;

$R_4$  is hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue; with the further proviso that at least one of  $R_1$  and  $R_2$  is either a glycosidic residue or an orthoester glycoside moiety.

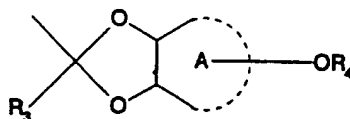
2. A method for alleviating the symptoms of menopause in an individual undergoing or having undergone menopause, comprising administering to said individual an effective amount of a compound having the formula:

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wherein  $R_1$  and  $R_2$  are independently hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue, or  $R_1$  or  $R_2$  is an orthoester glycoside moiety of the formula:

5



wherein A represents a glycofuranosyl or glycopyranosyl ring;

$R_3$  is hydrogen, lower alkyl ( $C_1-C_4$ ), aralkyl ( $C_7-C_{10}$ ), or aryl, with the proviso that aryl is phenyl or phenyl substituted by chloro, fluoro, bromo, iodo, lower  $C_1-C_4$  alkyl,  $C_1-C_4$  alkoxy; or naphthyl;

10

$R_4$  is hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue; with the further proviso that at least one of  $R_1$  and  $R_2$  is either a glycosidic residue or an orthoester glycoside moiety.

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3. The method of claim 1 or 2, wherein said compound is 17 $\beta$ -estradiol-3-( $\beta$ -glucoside).

4. The method of claim 1 or 2, wherein said compound is administered in an amount ranging from about 0.01 to 10 milligrams/kg per day.

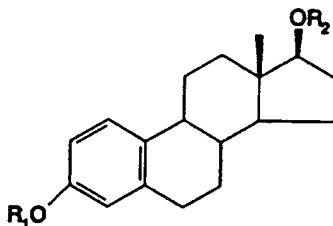
-17-

5. The method of claim 1 or 2, wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

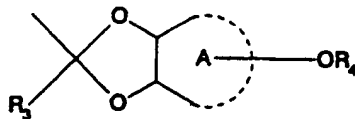
6. The method of claim 1, wherein said individual is suffering from or has suffered from menopause.

7. The method of claim 1 or 2, wherein said compound is administered to a woman prior to the onset of menopause.

8. A compound having the formula:



wherein R<sub>1</sub> is hydrogen, a straight or branched chain glycosidic residue containing 1-20 glucosidic units per residue, or an orthoester glycoside moiety of the formula:



wherein A represents a glycofuranosyl or glycopyranosyl ring;

R<sub>3</sub> is hydrogen, lower alkyl (C<sub>1</sub>-C<sub>4</sub>), aralkyl (C<sub>7</sub>-C<sub>10</sub>), or aryl, with the proviso that aryl is phenyl or phenyl substituted by chloro, fluoro, bromo, iodo, lower C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkoxy; or naphthyl;

R<sub>4</sub> is hydrogen or a straight or branched chain glycosidic residue containing 1-20 glycosidic units per residue; and

$R_2$  is an orthoester glycoside residue having the above formula, or a hydrogen when  $R_1$  is an orthoesterglycoside moiety.

9. The compound of claim 8 which is 3-estradiol-17 $\beta$ -( $\alpha$ -D-glucopyranosyl-1',2'-orthoacetate).

5           10. The compound of claim 8 which is 17 $\beta$ -estradiol-3- $\beta$ -glucopyranosyl-1',2'-orthoacetate.

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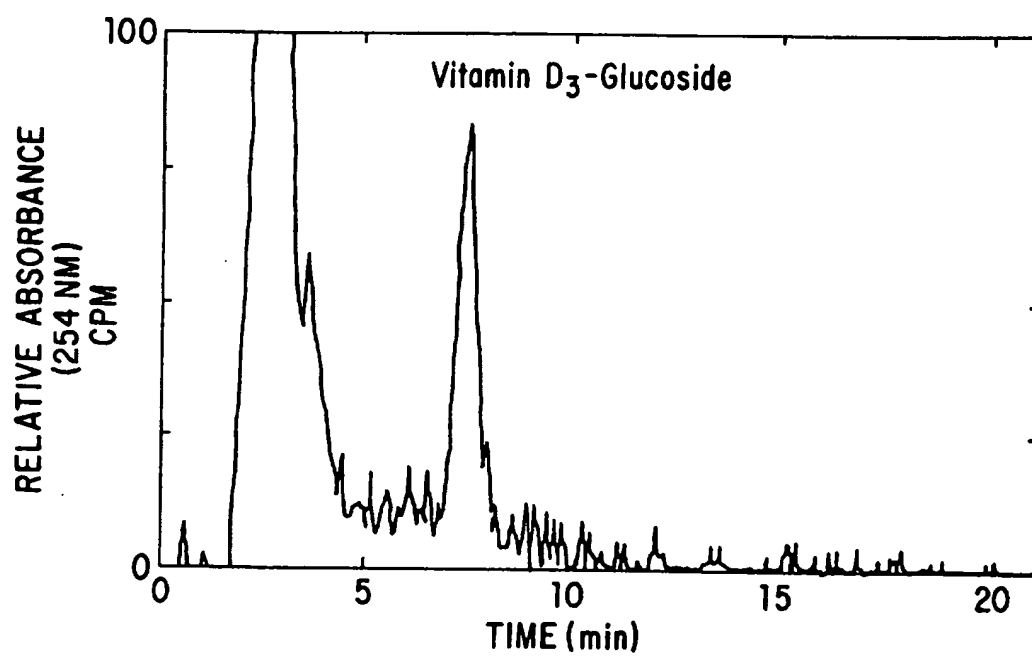


FIG. 1A

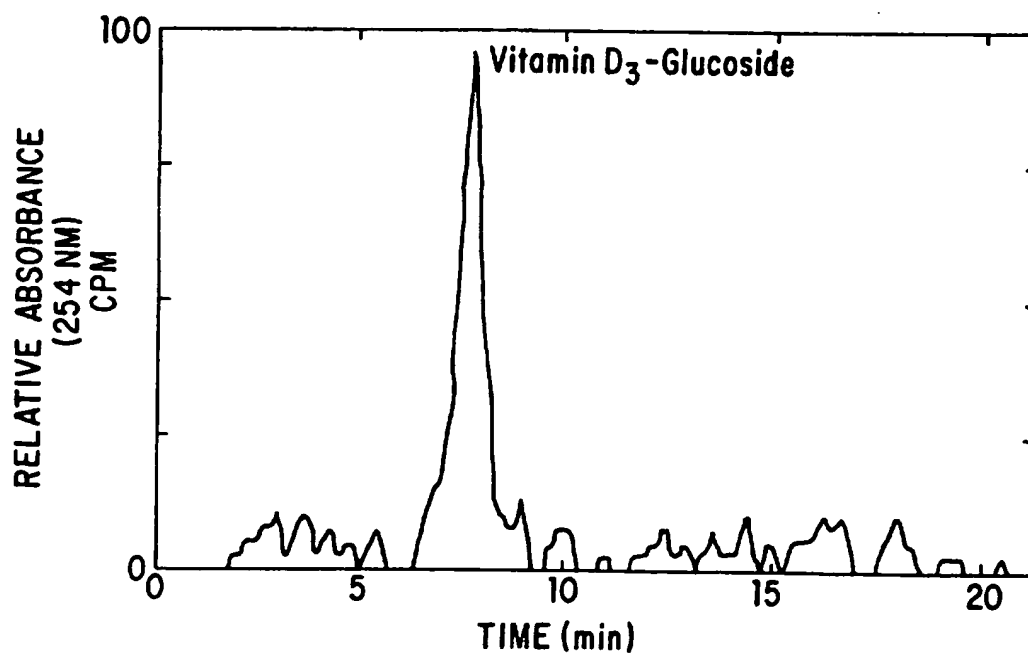


FIG. 1B

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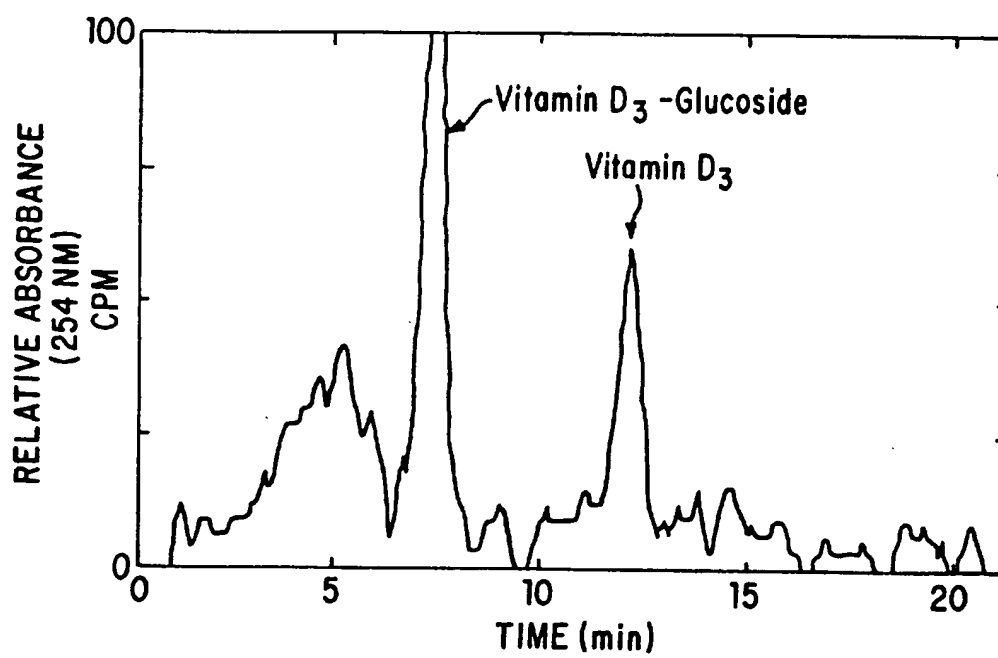


FIG. 1C

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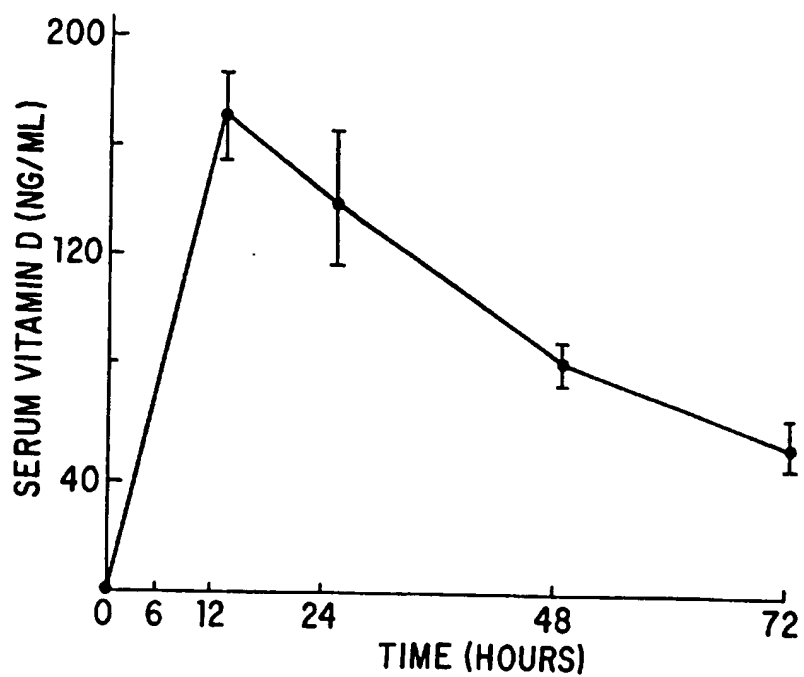


FIG. 2A

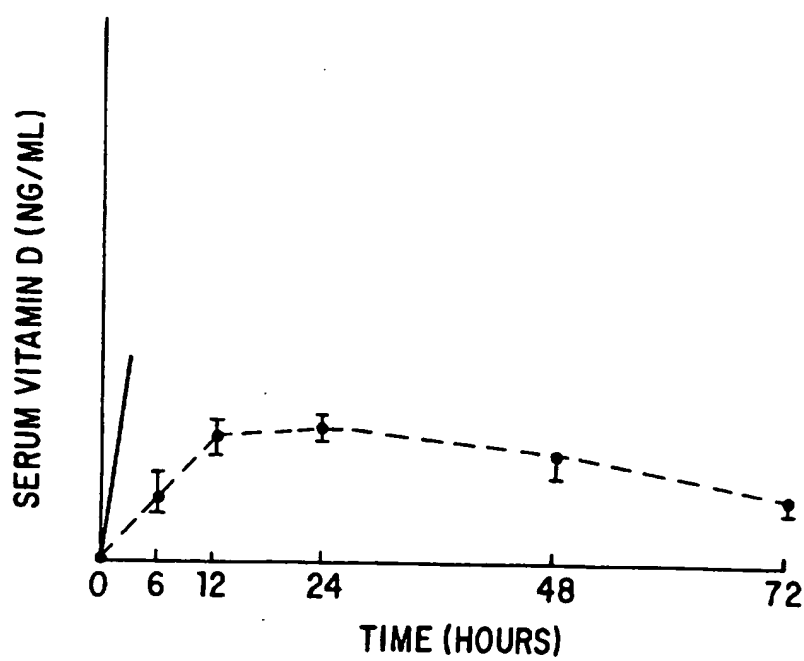


FIG. 2B

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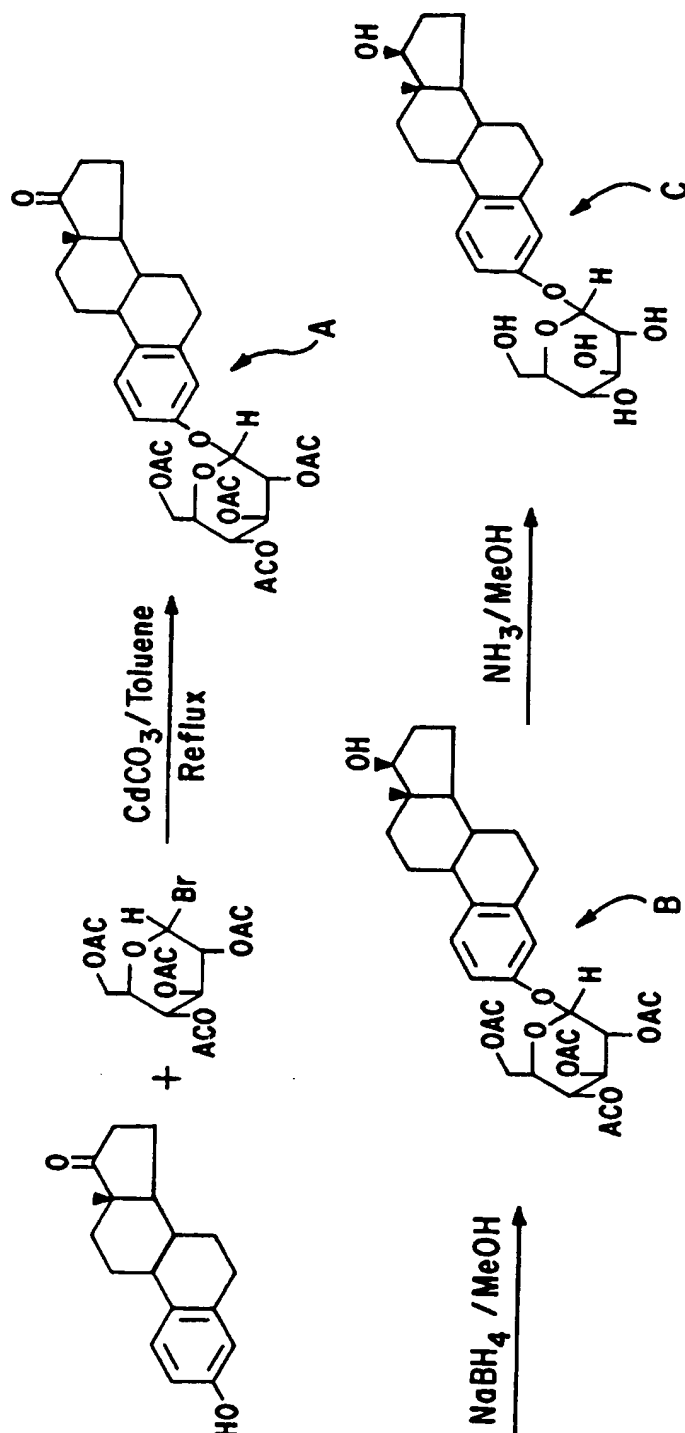


FIG. 3



## INTERNATIONAL SEARCH REPORT

 International application No.  
 PCT/US95/09720

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/705; C07J 1/00

US CL : 514/26; 536/5; 552/502

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/26; 536/5; 552/502

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

HCAPlus, BIOSIS, MEDLINE, APS

search terms: menopause, osteoporosis, estradiol (50-28-2)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Biochemistry, Volume 8, Number 11, issued November 1969, Williamson et al., "Isolation of 17 $\alpha$ -Estradiol 17- $\beta$ -D-Glucopyranoside from Rabbit Urine, and Its Synthesis and Characterization", pages 4299-4304, see entire document.	8-10 ---- 1-7
X --- Y	Experientia, Volume 42, issued 1986, Fujimoto et al., "Characterization of 17 $\beta$ -estradiol 3-( $\beta$ -D-glucopyranoside) and 17-( $\alpha$ -D-glucopyranoside) as the metabolites of 17 $\beta$ -estradiol in the cultured ovaries of the silkworm, <i>Bombyx mori</i> ", pages 567-568, see entire document.	8-10 ---- 1-7
Y	Gilman et al., "Goodman and Gilman's The Pharmacological Basis of Therapeutics", Eighth Edition, published 1990 by Pergamon Press, Inc. (Maxwell House, Fairview Park, Elmsford, NY), pages 1390-1395, see entire document.	1-7

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A		document defining the general state of the art which is not considered to be of particular relevance
* E		earlier document published on or after the international filing date
* L		document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
* O		document referring to an oral disclosure, use, exhibition or other means
* P		document published prior to the international filing date but later than the priority date claimed
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	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search

11 OCTOBER 1995

Date of mailing of the international search report

25 OCT 1995

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 Commissioner of Patents and Trademarks  
 Box PCT  
 Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

HOWARD C. LEE

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/09720

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,078,060 (BENSON ET AL.) 07 March 1978, see entire document.	1-10
A	US, A, 4,096,254 (BENSON ET AL.) 20 June 1978, see entire document.	1-10
A	US, A, 5,183,814 (DUKES) 02 February 1993, see entire document.	1-10